Choosing a mouse model to study the molecular pathobiology of Alport glomerulonephritis

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Alport syndrome, caused by mutations that interfere with the normal assembly of the α3α4α5(IV) collagen network in the glomerular basement membrane (GBM), is the most common inherited glomerular disease leading to renal failure. A detailed knowledge of the underlying pathogenic mechanisms is necessary for developing new, more specific, and effective therapeutic strategies aimed at delaying the onset and slowing disease progression. Studies of several dog and mouse models of Alport syndrome have significantly enhanced our understanding of the disease mechanisms and provided systems for testing potential therapies. In the most widely used *Col4a3*−/− mouse models of autosomal-recessive Alport syndrome (ARAS), the genetic background strongly affects renal survival. One contributing factor may be the strong ectopic deposition of α5α6(IV) collagen in the GBM of *Col4a3*-/- mice on the C57BL/6J background, which is almost undetectable on the 129/Sv background. This isoform 'switch' has not been observed in human ARAS, although it had been reported in the dog model of ARAS. In human patients as well as dog and mouse models of X-linked Alport syndrome, the α3–α6(IV) collagen chains are absent from the GBM. These biochemical differences among Alport animal models provide an opportunity to determine how the molecular makeup of the GBM affects the glomerular function. At the same time, potentially confounding influences of characteristics unique to a particular strain or model should be carefully considered in the design of studies aiming to define key events underlying the pathobiology of Alport glomerular disease.

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The glomerular basement membrane (GBM) is an important component of the glomerular filtration barrier. Its molecular composition changes during glomerulogenesis. The embryonic GBM contains a collagen IV network comprised of only α1(IV) and α2(IV) chains. A developmental switch is activated at the capillary loop stage of glomerular development, resulting in expression and assembly of α3(IV), α4(IV), and α5(IV) chains (Figure 1).[1–3] In mature glomeruli, the GBM consists of a relatively thin subendothelial α1α2(IV) collagen network and a relatively thick subepithelial α3α4α5(IV) collagen network. Alport syndrome is caused by mutations in the *COL4A3* or *COL4A4* genes (in the autosomal form of disease), or in the *COL4A5* gene (in the X-linked form), whereby functional inactivation of the gene, mRNA, or protein leads to the defective assembly and often the complete absence of all three chains in the GBM.[4] Instead, the embryonic α1α2(IV) collagen is deposited throughout the thickness of the mature Alport GBM. This different molecular composition of the Alport GBM is the underlying defect that somehow leads to the initiation of glomerular disease during childhood or adolescence. Before this unknown triggering event, the Alport GBM is slightly thinner than normal. During this time there is no measurable proteinuria and patients have a normal glomerular filtration rate. Onset of the disease is characterized by progressively increasing proteinuria associated with irregular thickening and splitting of the GBM and podocyte foot process effacement. Because the GBM functions normally in Alport patients for a time, it seems possible that blocking the event(s) that trigger disease initiation might arrest the disease in its pre-pathogenic state. Thus, identification of the events initiating disease, as well as those driving disease progression, is central issue of current research efforts. Nearly all of this work is being carried out using animal models.

Several spontaneous dog models for Alport syndrome have been identified and characterized. The earliest described and most studied is a model of X-linked Alport syndrome in a Samoyed breed containing a stop codon in exon 35 of the

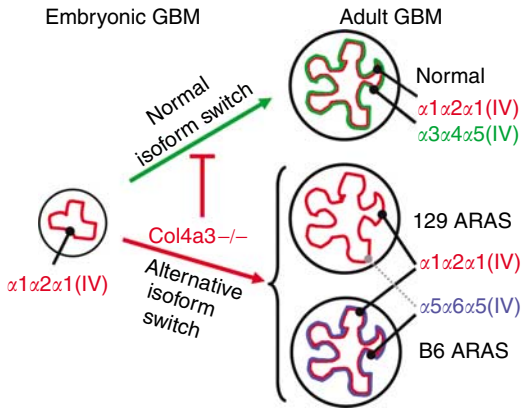

Figure 1 | Schematic diagram summarizing strain-dependent differences in type IV collagen isoform switches in ARAS. Normal embryonic GBM contains only α1α2(IV) collagen networks (red). At the capillary loop stage of development in mice, a developmental switch activates expression of the *COL4A3*, *COL4A4*, and *COL4A5* genes, resulting in the assembly of a subepithelial collagen network of α3α4α5(IV) chains (green), with a persistence of a subendothelial network of α1α2(IV) chains (red). In the *COL4A3*−/− mouse on the C57BL/6 background, an alternative isoform switch results in robust expression of α5α6(IV) networks in the GBM (blue). One report suggests very weak expression of the α5α6(IV) network in the COL4A3−/− mouse on the 129 background (dashed line).[12]

COL5A5 gene.[5] A second X-linked Alport model has been described in a mixed breed of dogs from Navasota, Texas.[6] Both of these models lack α3(IV), α4(IV), and α5(IV) collagen chains in their GBM. A canine model for autosomal-recessive Alport syndrome (ARAS) has been described; however, the mutation has not yet been identified.[7] In this model, α3(IV) and α4(IV) collagen chains are missing from the GBM, yet α5(IV) collagen is present (albeit at reduced levels compared with normal littermates). Importantly, α6(IV) collagen, which in normal kidneys is found only in the basement membranes of the Bowman's capsule and collecting ducts, is ectopically present in the GBM of these dogs. Expression of α6(IV) collagen had never been reported in the GBM of any mammalian species, normal or diseased. End-stage renal failure in these dogs occurs somewhat later (12–18 months) than either the Samoyed model (8–10 months) or the Navasota model (10–15 months). However, it is not known whether this is linked to the deposition of α5/α6(IV) collagen in the GBM of this model.

Several mouse Alport models have also been described. Two models for ARAS have been developed by gene targeting at the *Col4a3* locus.[8,9] Both were initially characterized in a mixed background, and have a similar pathologic profile in the glomerulus. One of these models is commercially available through the Jackson Laboratories on the 129/SvJ background. More recently, a murine model for X-linked Alport syndrome has been generated by introducing a G5X mutation in the *Col4a5* gene; these mutant mice are on the C57BL/6 background.[10] In the original description of these different mouse models, all of them were reported to lack expression of α3(IV), α4(IV), and α5(IV) collagen chains in the GBM. The influence of the genetic background on the timing of the onset of glomerular disease and the rate of progression to end-stage renal failure was studied by backcrossing autosomal Alport mice onto both the 129/SvJ and the C57BL/6J backgrounds.[11] C57BL/6 *Col4a3*−/− mice, compared with 129 Alport mice showed a markedly delayed disease onset (measurable proteinuria at 100 versus 35 days) and end-stage renal failure (194 versus 66 days). After backcrossing F1(129 × B6) hybrid mice to the parental C57BL/6 strain, DNA from the resulting N2 mice with fastest and slowest rates of renal progression was used in mapping studies, identifying two quantitative trait loci that segregate with the two extremes.[11] The putative modifier loci were located on chromosomes 9 and 16, but the responsible genes have not yet been identified.

Very recently, strain-dependent differences in the GBM composition of *Col4a3*−/− mice were identified that may also affect the rate of progression to renal failure.[12] Slow-progressing C57BL/6 *Col4a3*−/− mice exhibited robust GBM deposition of the α5(IV) collagen chain accompanied by the ectopic α6(IV) collagen. The two chains were biochemically associated with each other and with α1(IV) and α2(IV) collagen, indicating co-assembly as an α1α2/α5α6(IV) collagen network (Figure 1). Much lower amounts of α5(IV) and α6(IV) collagen were detected in the GBM of 129/Sv *Col4a3*−/− mice; this was attributable to an overall low synthesis or defective assembly of α6(IV) collagen-containing networks in tissues from 129/Sv mice, independent of the Alport mutation. In reciprocal F1(B6x129) crosses of *Col4a3*−/− mice, it was found that strong GBM deposition α5/α6(IV) collagen was inherited in a pattern consistent with X-linked transmission, and it correlated with a 46% increase in the longevity observed in C57BL/6 versus 129/Sv affected mice.[12] By comparing the GBM composition and renal survival in *Col4a3*−/− mice on pure and hybrid backgrounds, it was estimated that the ectopic α5α6(IV) collagen in the Alport GBM contributes about 28% of the added lifespan observed in C57BL/6 versus 129/Sv affected mice.

In contrast to observations in mouse and dog models of ARAS, immunochemical studies of collagen IV chains in the GBM of human patients with this form of disease have not identified ectopic expression of α6(IV) collagen, nor the presence of α5(IV) collagen.[13,14] The variable expression of α5/α6(IV) collagen in the autosomal-recessive Alport GBM – ranging from strong in mutant B6 mice to moderate in affected dogs, very weak in 129 mutant mice, and absent in human patients raises the question of how this network affects the glomerular function, the onset of disease, and its rate of progression. The physico-chemical properties and biological functions of extracellular matrices are determined by their molecular makeup, and changes in composition likely affect both cell adhesion and cell signaling. Because the properties of the embryonic α1α2(IV) collagen network are distinct from those of the adult α3α4α5(IV) network, it is likely that the persistence of the embryonic α1α2(IV) collagen

network in the GBM of Alport patients has multiple consequences that culminate in glomerular disease initiation. Fewer inter-chain cross-links in the α1/α2(IV) collagen network[15] likely affect its elasticity as well as susceptibility to endoproteolytic cleavage,[1] which may promote its eventual breakdown. Furthermore, it has been shown that ectopic laminins are deposited or re-deposited in the GBM of both 129/Sv and C57BL/6J autosomal Alport mice.[16–18]

Alterations of GBM composition likely contribute to physical changes in the podocytes that lead to foot process effacement and loss of the glomerular filtration barrier. As podocytes normally adhere to the GBM containing α3/α4/α5(IV) collagen, it is likely that podocytes might be distinctively influenced by the presence of α1/α2(IV) in the Alport GBM, which in turn may have different effects than ectopic α5/α6(IV) collagen. Earlier studies have shown altered gene expression in Alport glomeruli compared with wild-type glomeruli. Podocyte-specific induction of mRNA encoding transforming growth factor-α1 and matrix proteins has been shown,[19] and inhibition of transforming growth factor-α1 results in reduced thickening of the GBM.[18] More recently, podocyte-specific induction of CCR2 and matrix metalloproteinase-12 was demonstrated to contribute to the GBM destruction associated with Alport syndrome.[20] All of these studies were performed in the 129/Sv autosomal Alport mice and validated using necropsy specimen from human Alport patients. It is possible that some of these observations might not be recapitulated in C57BL/6 Alport mice. A comparison of two recent studies using different strains of *Col4a3*−/− mice underscores this risk. One study performed in 129/Sv Alport mice documented no change in glomerular expression of matrix metalloproteinase-2.[20] The other study, conducted in C57BL/6 Alport mice documented a significant elevation of glomerular matrix metalloproteinase-2 expression.[21] Thus, the results likely reflect strain-specific differences in the regulation of a potentially important modulator of glomerular pathogenesis.

The use of the C57BL/6 background does have certain advantages. First, slower progression allows a wider window for studying events associated with the initiation of glomerular pathology. It also allows colony maintenance by homozygous breeding, reducing the number of animals and costs. Second, many knockout mouse strains carrying mutations in genes of interest to investigators studying Alport glomerular pathogenesis are in the C57BL/6 background, which simplifies the production of purebred mice with multiple knockout mutations. Third, the availability of both autosomal-recessive and X-linked Alport mice on the C57BL/6 background, expressing different collagen IV networks in the mutant GBM, affords investigators a choice of models with 'customized' GBM composition.

Finally, the existence of genetic modifiers documented through the mapping of quantitative trait loci provides a powerful incentive to study the C57BL/6 Alport mouse models. In both X-linked and autosomal Alport mice on the C57BL/6 background, the onset of proteinuria is not observed until adulthood, whereas it occurs at the pre-pubescent stage of life in all Alport dog models and in 129/Sv Alport mice, as in human patients. Identification of the genes responsible for the delayed onset of disease would provide meaningful insights into understanding the molecular events underlying glomerular pathogenesis in Alport syndrome, and may provide the basis for developing novel therapeutic strategies. On the other hand, basic studies aimed at defining specific molecular pathways influencing glomerular disease initiation and progression might be affected unpredictably by the modifier effects, whereas the duration and expense of studies testing novel therapeutic agents in C57BL/6 Alport mice would be considerably increased.

All of us interested in exploring the mechanisms of Alport glomerular pathogenesis aim to identify pathways relevant to the human disease. In the coming years, it is likely that exploitation of the existing animal model systems will yield a wealth of information toward this end. If we do not choose our model systems carefully, we run the risk of creating an inchoate body of literature. This would surely hamper the development of effective therapeutic strategies for what should be a treatable disease.

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